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HAZLETON LABORATORIES

INCORPORATED • FALLS CHURCH, VIRGINIA

EVALUATION OF NATURAL PRODUCTS

FINAL REPORT

EDGEWOOD ARSENAL CONTRACT NO.:
DA 18-108-AMC 255 (A)

This report summarizes the progress made for the period July 1, 1963 through April 30, 1965.

Approved by:

OTHO D. EASTERDAY, Ph. D.
Principal Investigator

HAZLETON LABORATORIES, INCORPORATED

Falls Church, Virginia

Unclassified

AD_____Accession No._____ Unclassified

Hazleton Laboratories, Inc., Falls Church, Virginia.

EVALUATION OF NATURAL PRODUCTS -
O. D. Easterday and I. Cornman

Final Report, July 1, 1963 through April 30, 1965, 15 pp - 2 tables - 2 plates
Contract No. DA-18-108-AMC (A)

Final report on the Field Screen and Primary Screen for the evaluation of natural products. Forty-four materials of the 1174 studied were recommended for testing in the Secondary Screen. It was recommended that five natural products be further purified and the active principle isolated.

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Toxicity
Natural Products
Plants

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Several different research and development rationales have been discussed for the discovery of therapeutic and CBR agents. An investigator may follow any one of these in seeking new materials for beneficial or destructive purposes. A choice exists between the following fundamental approaches: (1) the chemical synthesis of new molecular species or (2) the revealing of potent materials found in natural products. The conceptual application of random and non-random approaches in relation to these two choices has been considered using well-known drugs as examples in each case. The relative merits and difficulties of the two choices and two approaches are extensively discussed.

In a recent review of "The Pharmacopoeia" made as a part of this program, it was found that 238 of the drugs (not monographs) listed were derived from natural products and 216 drugs had their origin as synthetic chemicals. Considerable argument was presented regarding the relative value of the search for new agents by means of chemical synthesis or by the discovery and evaluation of natural products. It was concluded there should exist no categorical argument of "either - or". Nature unquestionably has provided many useful potent drugs which have been developed through natural products research and many agents undoubtedly still wait to be discovered in the great quantity of materials which have not yet been systematically examined. These unexplored materials, in the light of past discoveries, should provide a great incentive for the necessary chemical synthesis and natural product research which remains to be performed.

The annual reports discussed in considerable depth the principles followed, the techniques used, the results obtained, and the advantages for the natural products research program. This program was composed of a Field Screen and a Primary Screen. The Field Screen made possible the rapid testing of freshly collected specimens while the Primary Screen undertook the additionally required evaluation of selected specimens obtained from the Field Screen before these were further studied in the Secondary Screening Program.

The Field Screen encompassed these fundamental requirements of natural products screening: (1) a rapid procurement of specimens; (2) an immediate testing of samples before deterioration can occur; (3) a minimum difficulty in the shipment of materials; (4) elimination of the problems that attend the use and provision of mammals in preliminary screening as the areas for collection change; and (5) reduction of the difficulties of testing by using animal species available locally at the test site.

The Field Screen tests were made on aqueous, acetone, dimethylformamide and/or ethanol extracts of the collected specimens in toto or their various parts. The systems employed were (1) the eggs of a sea

urchin (Lytechinus variegatus), (2) the mosquito fish (Gambusia affinis), (3) the brine shrimp (Artemia salina), (4) fragments of a sea urchin (Diadema antillarum), (5) germinating seeds (Brassica, mustard and Secale cereale, rye), and (6) microorganisms (Aspergillus niger, Staphylococcus aureus, Eschericha coli, and Bacillus subtilis). The principles, details of the procedures, and the observations which were made and recorded were presented for each test system in the monthly and annual reports. All of the related natural product information was summarized in tables.

A total of 1174 natural products were investigated in the two programs, of which 1049 were studied in the Field Screen. Of these, 234 were examined by additional follow-up studies in the Field Screen. There were 3582 plant or animal parts studied. A total of 8061 aqueous, acetone, dimethylformamide, or ethanol extracts were evaluated and 79,507 individual tests or observations were made. The number of positive responses for each system or sub-system were as follows*:

1. Sea Urchin Eggs
   a. Retardation - 1007
   b. Blocking - 1707
2. Fish
   a. Behavioral - 159
   b. Death - 719
3. Brine Shrimp - 422
4. Nerve-muscle - 121
5. Seeds
   a. Mustard - 823
   b. Rye - 661
6. Microorganisms
   a. A. niger - 95
   b. S. aureus - 181
   c. E. coli - 87
   d. B. subtilis - 230

The number of extracts giving positive responses in any one, two, three, four, or five combinations or in all six systems were: one system - 1448, two systems - 866, three systems - 393, four systems - 164, five systems - 45, and six systems - 12*.

Of the 1049 natural products studied, 204 (19%) were described in narrative discussions and 137 (13%) were forwarded and studied in the Primary Screen.

Also investigated in the Field Screen were 58 reference pharmaceuticals as listed in Table No. 1. It was observed that the distribution of the positive responses among the six systems was more uniform than that

* These data are incomplete, due to the unavailability of some of the unreported data.

observed with the natural products. The number of solutions active in the system combinations showing positive responses tended to be larger - particularly for the three and larger system combinations. These results have suggested that the profiles which may be formed are of major significance. These profiles may have useful predictive value in the selection of successful candidate drugs.

The Primary Screen comprised the preparation of two solvent extracts (aqueous and ethanol) for each natural product and the intravenous or intraperitoneal administration of these extracts to mice employing a pharmacotoxic experimental procedure. The natural products studied were received from the Field Screen Program and from other sources - Edgewood Arsenal, Research Triangle Institute, and other laboratories. The experimental procedures (extraction and biological assay) were described in detail in the monthly and annual reports.

A total of 280 natural products were studied. The Field Screen Program forwarded 137 natural products for which 278 plant or animal parts were evaluated. A total of 559 extracts (aqueous, ethanol, aqueous-ethanol) on specimens received from all sources were administered intravenously or intraperitoneally to mice. The number of natural products and extracts recommended for the Secondary Screen were 44 and 56, respectively. These were selected employing the criteria of (1) 3 mg/kg or less for the ED_{50}, (2) 10 mg/kg or less for the LD_{50}, (3) a combined application of the LD_{50}/ED_{50} ratio of 10 or greater with the ED_{50} and/or LD_{50}, and (4) unique pharmacotoxic signs. The N.P.O. numbers for the natural products and extracts selected for and transferred to the Secondary Screening Program are listed in Table No. 2. The selection criteria for each are also tabulated.

Thirteen natural products (14 extracts; eight ethanol, five aqueous, one aqueous-ethanol) were recommended for the Secondary Screen on the basis of their ED_{50} values, and 30 (38 extracts; 20 ethanol, 18 aqueous) were selected because of some unique pharmacotoxic property. Three natural products (four extracts; two ethanol, one aqueous, one aqueous-ethanol) were selected on the basis of both the ED_{50} and pharmacotoxic signs. The ethanol extraction procedure contributed the largest number of fractions.

An investigation of the relationship between particle size and possible embolism formation was made in mice. The conclusion was drawn that an oil-water emulsion might be given in small doses without seriously occluding the pulmonary circulation. The injection of latex particles demonstrated that the pulmonary capillaries permitted passage of particles up to 10 to 12 micra in diameter. The injection of

N.P.O. No. 715,001 produced fatal occlusion of pulmonary arteries by masses of the suspended material. The injection of N.P.O. 715,042 apparently caused pulmonary arterial occlusion due to the inability of the injection mass to pass through the alveolar capillaries.

Another study was undertaken to obtain control dose-reponse data and pharmacotoxic observations on the various solutes (95% ethanol, 0.5% methylcellulose, PEG 200, propylene glycol, saline, and sterol solvent) used for the solubilization or suspension of the extracts to be injected. The observed LD_{50} and ED_{50} estimates for the intravenous and intraperitoneal routes of administration have been included.

The effect of centrifugation upon the pharmacotoxic data was investigated. These experimental data suggested that centrifugation removed a biologically active component from the extract since the intraperitoneally administered centrifuged supernatant fractions, in all cases, were characterized by having an increased LD_{50} dose.

Two dosage schedules were examined in order to determine effects upon the pharmacotoxic data and the LD_{50}/ED_{50} ratio. One identified as Schedule A was the preferred progression particularly with reference to whether or not a false positive selection of a natural product would be made because of the actual ED_{50}, LD_{50}, and their ratio values.

A special study was performed to obtain pharmacotoxic experience and data for a series of reference pharmaceuticals. The materials investigated are listed in Table No. 1.

Another special study was done on the Natural Product, N.P.O. No. 730,141, forwarded by Edgewood Arsenal to Hazleton Laboratories. This material was administered to two mice intravenously, intraperitoneally, percutaneously, ocularly, and intravaginally. Solutions were prepared daily of the various concentrations used. The product was dissolved in sodium hydroxide and distilled water.

This natural product was investigated in mice for its irritant, pharmacotoxic, and lethal properties. In mice, at 20.0 mg/kg, total mortality was observed within 24 hours following intravenous administration. One animal succumbed at a 10.0 mg/kg dosage level. Pharmacotoxic and autopsy observations were recorded. Following intraperitoneal administration, at the 20.0 mg/kg dosage level, toxicity signs were observed. Pharmacotoxic effects were not observed at any of the dosage levels following percutaneous, ocular, and intravaginal administration. With these routes no gross pathology was noted.

This material in the ileal strip preparation and at a dosage level of 100 ug/ml (final concentration) produced a 22% inhibition of the acetylcholine response. A dose of 46.4 ug/ml produced no response. The doses, 100 and 46.4 ug/ml for the natural product did not have an independent relaxation or contraction effect on the ileum strip as a direct action of the drug itself.

A special study was done to determine if the use of a 10-solvent sequential extraction system would isolate and partially purify the efficacious substance found in the previously tested and active natural products. The following Natural Products were investigated:

N.P.O. No.	N.P.O. No.
715,010	715,462 (bark)
715,090	715,462 (mature)
715,121	715,499
715,401	715,501

These seven natural products, producing 80 fractions and 91 resumes, were evaluated employing a slightly modified pharmacotoxic procedure and has been described in detail in the annual reports. Of the fractions studied the following were observed to be more active than the others:

N.P.O. No.	Fractions
715,010	Diethyl ether, ethanol (hot), water
715,090	Chloroform, methanol, water, water (hot)
715,121	Chloroform, ethanol, ethanol (hot)
715,401	Water
715,501	Diethyl ether, chloroform

This procedure produced fractions having a several fold increase in activity; for example, N.P.O. No. 715,010 had a previous detectable activity at 31 mg/kg for the aqueous fraction and with this technique it was active at 0.25 mg/kg. For N.P.O. No. 715,090 the detectable activity for the ethanol fraction was increased from approximately 73 to 5.0 mg/kg. The aqueous fractions activity remained comparable. The potency for N.P.O. No. 715,121 was, in general, approximately tripled.

The data, obtained from a marine biological program supported by funds other than those contracted for in this program, were released to Edgewood Arsenal for review and utilization. These data were divided into two categories: (1) those previously screened with pharmacology and toxicology follow-up, and (2) those previously screened with no

pharmacology or toxicology follow-up. The latter materials were investigated as a component part of the Edgewood Arsenal Primary Screening program and include N.P.O. No. 715,460 to N.P.O. No. 715,524 (inclusive). The data for these latter materials were reported in the monthly reports in the on-site experimental section. The data for the previously screened materials were reviewed and reported under support by Edgewood Arsenal and were reported as special studies. The natural products included were N.P.O. No. 715,401 to N.P.O. No. 715,459 (inclusive). The following discussion is related to the previously evaluated materials, studied approximately three years ago.

During these investigation, other techniques were performed beyond those specified in the Edgewood Arsenal contract. Different protocol was used; therefore, the previously obtained data were considered as a special study for report purposes. The data for 53 natural products were reviewed. The biological assays included the following procedures: microbiological; mouse, cat, and rabbit acute pharmacotoxic; repeated rat oral toxicity; rat, dog, and cat pharmacodynamic; and antitumor. However, all of these procedures were not used on each natural product; only those procedures which were indicated by the kind of field screen activity were used in the on-site studies.

One natural product, N.P.O. No. 715,401, observed to have interesting activities and potencies in the off-site screening program was further evaluated in an expanded on-site screening program. The on-site evaluative methods were microbiological; rat, cat, mouse, and rabbit pharmacotoxic; and dog, cat, and rat pharmacodynamic. Natural Product N.P.O. No. 715,401 appears to be a hypotensive agent characterized by having a long sustained duration of action, not destroyed by enzymatic action (orally active), nor affected by heat (thus not a protein) and is stable to harsh chemical treatment. Chemical and biological data confirm the conclusion that this material is not a protein but more likely a poly-macromolecule of medium molecular weight. The material does not appear to have any anticholinesterase activity; however, does inhibit certain of the neurohumoral responses.

The following natural products and their respective activities were found of interest.

N.P.O. No.	Activity of Interest
715,403	Microbiologic
715,407	Microbiologic
715,408	Microbiologic and pharmacotoxic (cat)
715,418	Pharmacodynamic, blood pressure effective dose, 4.7 mg/kg

N.P.O. No.	Activity of Interest
715,424	Pharmacodynamic, blood pressure effective dose, 3.2 mg/kg
715,426	Microbiologic and pharmacodynamic (cat) potency
715,428	Pharmacodynamic, blood pressure effective dose, 2.0 mg/kg
715,430	Pharmacodynamic, inhibition and blocking of neurohumors
715,437	Pharmacodynamic, blood pressure effective dose, 1.0 mg/kg, epinephrine response inhibited or blocked
715,439	Pharmacodynamic, blood pressure effective dose, 2.4 mg/kg

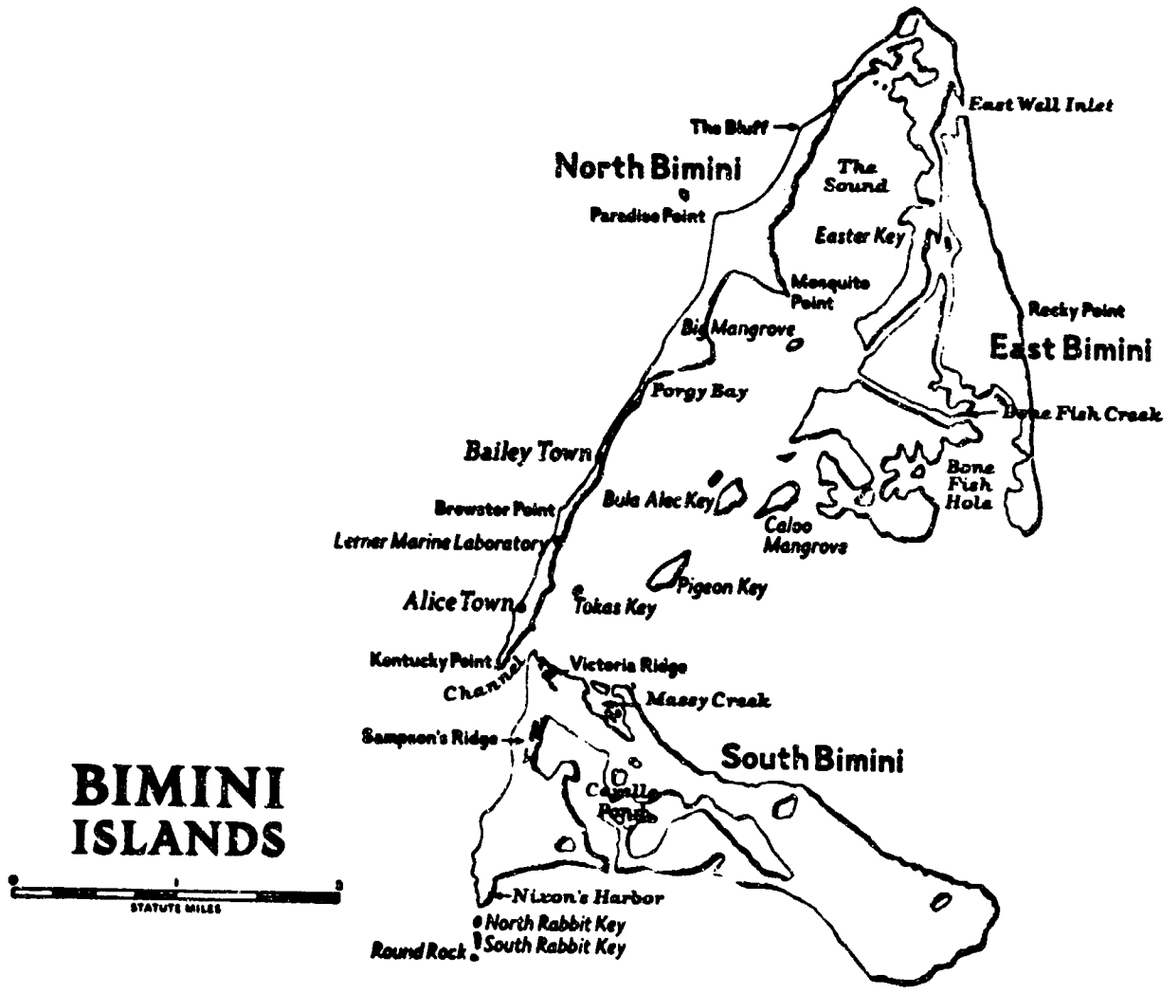
Thirty-two natural products were received from one of the collectors which have not been evaluated in the Field or Primary Screens. These materials should be examined at the earliest possible moment in order to prevent adverse changes from occurring.

A detailed examination is recommended of the correlation that exists between the data of the reference pharmaceuticals obtained with the various systems of the Field Screen and other known pharmacological information. This evaluation should provide a valuable insight as to the most useful property or properties of the Field Screen Methodology having predictive utility in the selection of active therapeutic and incapacitating drugs. Further follow-up is recommended with regard to the natural product candidates that enter the Secondary Screening Program. An examination is recommended which would closely review the Field and Primary Screen properties that formed the basis for the natural products selection for additional study. This would shed some knowledge upon those characteristics having the greatest value for rapid screening.

The most important of all of the recommendations that can be made is that additional isolation and purification should be immediately initiated with those natural products having a high potency in the Field and Primary Screens. It was demonstrated that the active material was concentrated in the three materials that were partially purified, thus an increased potency can be further achieved in the already highly toxic products.

Maps (pages No. 9 and No. 10) are included to show the general area where collections were made by Dr. Cornman. The specimens were collected on the shoreline vicinity and in different locales throughout Bimini and Jamaica and at various elevations. Also, the following bibliography is included:

1. Adams, C. D., Magnus, K., and Seaforth, C., Poisonous Plants in Jamaica, Extra - Mural Studies, U. of West Indies, June, 1963.
2. Asprey, G. F., and Thornton, P., Medicinal Plants of Jamaica, Part I, West Indian Medical Journal, 2, 233, 1953.
3. Asprey, G. F., and Thornton, P., Medicinal Plants of Jamaica, Part II, West Indian Medical Journal, 3, 71, 1954.
4. Asprey, G. F., and Thornton, P., Medicinal Plants of Jamaica, Parts III and IV, West Indian Medical Journal, 4, 69, 1955.
5. Britton and Millspaugh, Bahama Flora, Britton and Millspaugh, June 26, 1920.
6. Browne and Patrick, The Civil and Natural History of Jamaica, B. White and Son, Horace Head, Fleet Street, London, 1789.
7. _____, Bulletin of the Institute of Jamaica, Science Series, No. 12, Part I, 1961.
8. _____, Bulletin of the Institute of Jamaica, Science Series. No. 12, Part II, 1963.
9. Chapman, V. J., The Marine Algae of Jamaica, Institute of Jamaica.
10. Oakes, A. J., and Butcher, J. O., Poisonous and Injurious Plants of the U. S. Virgin Islands, Agricultural Research Service, U. S. Department of Agriculture, Miscellaneous Publication No. 882, April, 1962.


BIMINI
ISLANDS
STATUTE MILES
East Wall Inlet
The Bluff
North Bimini
The Sound
Paradise Point
Easter Key
Mosquito Point
Rocky Point
East Bimini
Big Mangrove
Porgy Bay
Bone Fish Creek
Bailey Town
Bone Fish Hole
Bula Alec Key
Brewster Point
Caloo Mangrove
Lerner Marine Laboratory
Pigeon Key
Alice Town
Tokas Key
Kentucky Point
Victoria Ridge
Channel
Massy Creek
Sampson's Ridge
South Bimini
Nixon's Harbor
North Rabbit Key
Round Rock
South Rabbit Key

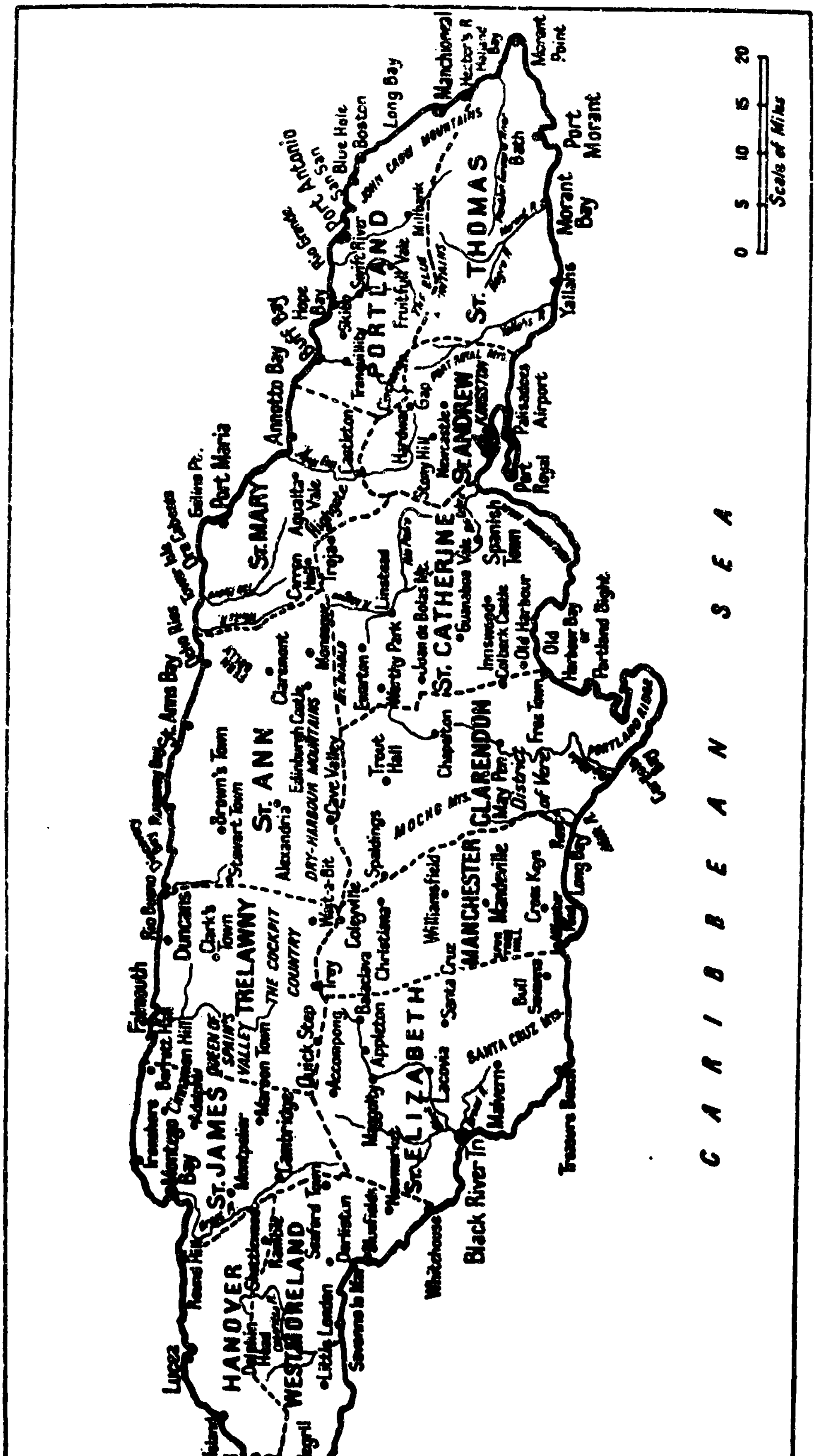

HANOVER
WESTMORELAND
ST. JAMES
TRELAWNY
ST. ELIZABETH
MANCHESTER
ST. ANN
CLARENDON
ST. CATHERINE
ST. MARY
ST. ANDREW
PORTLAND
ST. THOMAS
CARIBBEAN SEA
THE COCKPIT COUNTRY
DRY-HARBOUR MOUNTAINS
MOCHO MTS.
SANTA CRUZ MTS.
JOHN CROW MOUNTAINS
Lucea
Montego Bay
Falmouth
Duncans
Rio Bueno
St. Anns Bay
Port Maria
Annotto Bay
Port Antonio
Long Bay
Manchioneal
Morant Point
Port Morant
Morant Bay
Yallahs
Bath
Kingston
Palisadoes
Airport
Port Royal
Spanish Town
Old Harbour
Old Harbour Bay
Portland Bight
Black River
Whitehouse
Savanna la Mar
Negril
Green Island
Mandeville
Christiana
Spaldings
Linstead
Claremont
Brown's Town
Alexandria
Cave Valley
Chapelton
May Pen
Williamsfield
Santa Cruz
Malvern
Lacovia
Maggotty
Appleton
Balaclava
Accompong
Quick Step
Cambridge
Montpelier
Troy
Wait-a-Bit
Clark's Town
Stewart Town
Lime Hall
Bog Walk
Constant Spring
Stony Hill
Newcastle
Gordon Town
Buff Bay
Hope Bay
Fruitful Vale
Millbank
Blue Hole
Boston
Scale of Miles
0 5 10 15 20
E.G.M.

Table No. 1 - Pharmaceuticals Investigated

REFERENCE PHARMACEUTICAL	OFF-SITE	ON-SITE
Acetylcholine chloride	x	
Adrenaline chloride	x	
Ammonium molybdate	x	
Apomorphine	x	x
Atropine sulfate	x	
Barbital sodium	x	x
Barium chloride	x	
Chlorisondamine chloride*		x
Chlorpromazine hydrochloride	x	
d-Amphetamine sulfate	x	x
Dibenzyline hydrochloride	x	x
Dihydrostreptomycin	x	
Endrin	x	
Ephedrine sulfate	x	x
Epinephrine chloride (Adrenaline)	x	
Ergotamine	x	
Heparin sodium	x	
Hexamethonium chloride	x	
Histamine phosphate	x	
Imipramine hydrochloride	x	
Lead nitrate	x	
Librium	x	x
Mercury nitrate	x	
Methacholine chloride	x	
Methocarbamol	x	
Methoxyclor	x	
Methylatropinium bromide	x	x
Metrazol	x	
Nikethamide	x	x
l-Norepinephrine bitartrate	x	
Pentobarbital	x	
Phenobarbital sodium	x	
Physostigmine sulfate/salicylate**	x	x
Reserpine	x	x
Serotonin creatinine sulfate	x	x
Sodium fluoride	x	
Strychnine sulfate	x	x
Succinylcholine chloride*		x
Tetra ethyl ammonium chloride	x	
Tremorine dihydrochloride	x	x
Tubocurarine chloride	x	
Yohimbine hydrochloride	x	x

* These pharmaceuticals (chlorisondamine chloride and succinylcholine chloride) were not scheduled for off-site evaluation.

** Physostigmine sulfate used on-site, salicylate used off-site.

Table No. 1 - Continued

REFERENCE PHARMACEUTICAL	OFF-SITE	ON-SITE
BZ	x	x
CAR 302033	x	
CN	x	
CS	x	
CS 2245	x	x
CS 3246	x	
CS 3687	x	x
CS 4640	x	x
CS 4756	x	
CS 24064	x	x
DM	x	
EA 1476	x	x
EA 2148	x	x
EA 2233	x	
EA 2277		
EA 3443	x	
EA 3528	x	x
EA 3862	x	
EA 3867	x	
TOTAL 61	58	24

Table No. 2 - Summary of Natural Products, Parts, and Extracts Selected for and/or Transferred to the Secondary Screening Program.

N.P.O. NO.	PLANT OR ANIMAL PART	EXTRACTION SOLVENT	TRANSFERRED	ROUTE	DOSE LD_{50}	DOSE ED_{50}	SELECTION CRITERIA*	SOURCE	NATURAL PRODUCT REPORT NO.
700,090		EN	**	IP	266	2.9	1,3	RTI	10
700,121		EN	+	IV	>1000	51	4	RTI	10
700,125		EN	+	IP	600	11	4	RTI	10
700,450	BK	EN	+	IP	>40	2.5	1,3	RTI	21
700,543	BK	EN	+	IP	>125	2.8	1,3	RTI	21
715,010	FR	EN	**	IV	198	4.3	4	EA	7
715,015	FR & LF	EN	+	IV	>562	0.78(1.9)	1,3	EA	7
				IV	>5	1.8	1	EA	7
				IP	>562	3.1	1,3	EA	7
715,025	RT	AQ	+	IV	178	1.99	1,3	EA	7
				IV	7.8	2.8	1	EA	7
715,038	FL	EN	**	IP	>1650	<3.2	1,3	EA	7
715,042	FL	EN	+	IV	206	1.14	1,3	EA	7
				IP	>824	4.55	4	EA	7
715,060	LF & BK	AQ	**	IV	75	3.69	4	EA	7
715,071	LF	AQ	+	IV	375	11.7	4	EA	10
		EN	+	IP	486.3	15.2	4	EA	10
				IP	585	146	4	EA	10
715,074	LF & FR	AQ	**	IV	625	3.43	4	EA	7
715,090	LF	AQ	**	IV	20	0.375	1,3	EA	7
	RT	AQ	**	IV	125	1.4	1,3	EA	7
715,105	RT	AQ	+	IV	45.8	1.01	1,3	EA	8
715,121	LF & ST	EN	**	IP	89.4	3.95	4	EA	8
715,153	FL,LF,BK	AQ	+	IV	425	75	4	EA	8

* (1) ED_{50} $\leq$3.0 mg/kg; (2) LD_{50} $\leq$10.0 mg/kg; (3) LD_{50}/ED_{50} $\leq$10, this ratio was used in conjunction with one or both the preceding criteria and only when No. 1 or No. 2 suffice, the extract was transferred to the Secondary Screening Program. The ratio alone was not used to determine the transfer to the Secondary Screen. (4) Unusual and/or interesting pharmacotoxic signs.

** The supply of these extracts was depleted. There was no reserve supply available for transfer to the Secondary Screening Program.

Table No. 2 - Continued

N.P.O. NO.	PLANT OR ANIMAL PART	EXTRACTION SOLVENT	TRANSFERRED	ROUTE	DOSE LD_{50}	DOSE ED_{50}	SELECTION CRITERIA*	SOURCE	NATURAL PRODUCT REPORT NO.
715,185	SD	AQ	+	IV	32.2	16.5	4	EA	13
		EN	+	IP	63	15.0	4	EA	13
715,200	BK	EN	+	IP	708	2.7	1,3	EA	10
715,206	FL,LF,RT	AQ	+	IV	50	5.9	4	EA	10
715,231	LF	EN	+	IP	>500	2.7	1,3	EA	10
715,233	BK	EN	**	IP	>500	27.5	4	EA	12
	LF	AQ	+	IV	31.3	7.8	4	EA	12
715,244	SD	AQ	**	IV	530	34	4	EA	11
		EN	+	IP	>500	22.5	4	EA	12
715,256	FL	AQ	**	IV	62.5	5.5	4	EA	11
715,279	LF	EN	+	IP	355	2.0	1,3	EA	12
715,300	BK	AQ/EN	+	IP	176	1.4	1,3,4	EA	12
	FL	EN	+	IP	100	4.5	4	EA	13
	LF	EN	+	IP	530	1.0	1,3,4	EA	12
715,302	LF	AQ/EN	+	IP	500	2.8	1,3	EA	12
715,306	BK	EN	+	IP	285	3.1	4	EA	13
715,309	LF	AQ	+	IV	125	1.42	1,3,4	EA	13
		EN	**	IP	>750	32.2	4	EA	13
715,400	LF	AQ	+	IV	93.8	2.1	1,3	EA	20
715,462	FL	EN	+	IP	>400	17.8	4	FMC	FMC-1
	BK	EN	**	IP	353	5.5	4	FMC	FMC-1
	PL	EN	+	IP	>500	6.8	4	FMC	FMC-1
715,463	LF	AQ	+	IV	375	11.7	4	FMC	FMC-1
715,464	LF,ST	EN	+	IP	>500	5.5	4	FMC	FMC-1
715,465	LF	EN	+	IP	>500	22.5	4	FMC	FMC-1
715,466	RT	EN	+	IP	>400	14.5	4	FMC	FMC-1
715,470	RT	EN	+	IP	>500	48.5	4	FMC	FMC-1

* (1) ED_{50} $\leq$3.0 mg/kg; (2) LD_{50} $\geq$10.0 mg/kg; (3) LD_{50}/ED_{50} $\leq$10, this ratio was used in conjunction with one or both the preceding criteria and only when No. 1 or No. 2 suffice, the extract was transferred to the Secondary Screening Program. The ratio alone was not used to determine the transfer to the Secondary Screen. (4) Unusual and/or interesting pharmacotoxic signs.

** The supply of these extracts was depleted. There was no reserve supply available for transfer to the Secondary Screening Program.

Table No. 2 - Continued

N.P.O. NO.	PLANT OR ANIMAL PART	EXTRACTION SOLVENT	TRANSFERRED	ROUTE	DOSE		SELECTION CRITERIA*	SOURCE	NATURAL PRODUCT REPORT NO.
					LD_{50}	ED_{50}			
715,472	LF	AQ	+	IV	52.8	23.4	4	FMC	FMC-2
715,486	BK,LF	AQ	+	IV	62.5	3.9	4	FMC	FMC-3
715,487	PL	AQ	+	IV	355	7.8	4	FMC	FMC-2
715,498	TUNICATE	AQ	+	IV	46.9	4.2	4	FMC	FMC-3
		EN	+	IV	>500	22	4	FMC	FMC-3
715,501	AN	AQ	+	IV	5.9	0.28	1,3,4	FMC	FMC-4
		EN	+	IP	15.6	2.8	1	FMC	FMC-4
715,502	LF,PD	AQ	+	IV	167	2.0	1,3,4	FMC	FMC-3
715,506	AN	AQ	+	IV	167	5.5	4	FMC	FMC-3
715,513	LF	AQ	+	IV	62.5	5.5	4	FMC	FMC-5

* (1) ED_{50} $\leq$3.0 mg/kg; (2) LD_{50} $\leq$10.0 mg/kg; (3) LD_{50}/ED_{50} $\leq$10, this ratio was used in conjunction with one or both the preceding criteria and only when No. 1 or No. 2 suffice, the extract was transferred to the Secondary Screening Program. The ratio alone was not used to determine the transfer to the Secondary Screen. (4) Unusual and/or interesting pharmacotoxic signs.